过氧化物酶染色试剂盒(DAB 法)

货号： G2370
规格： 4×10mL/4×20mL
保存： -20℃, 避光保存, 有效期1年。

产品组成：

名称		4×10mL	4×20mL	保存
试剂(A): BFA 固定液		10mL	20mL	2-8℃, 避光
试剂(B): POX 孵育液	B1: DAB 染色液	10mL	20mL	-20℃, 避光
	B2: DAB 氧化剂	2×100μL	4×100μL	2-8℃, 避光
临用前，按 B1:B2=1000:1 比例混合，即为 POX 孵育液，即配即用。				
试剂(C): WG 染色液		10mL	20mL	室温, 避光
试剂(D): WG 缓冲液		10mL	20mL	室温

产品介绍：

过氧化物酶(Peroxidase，简称 POX 或 MPO)是由微生物或植物所产生的一类能催化很多反应的以过氧化氢为电子受体催化底物氧化的氧化还原酶，主要存在于细胞的过氧化物酶体中，以铁卟啉为辅基，可催化过氧化氢氧化酚类和胺类化合物，具有消除过氧化氢和酚类、胺类毒性的双重作用。

过氧化物酶染色液(DAB 法)是 ICSH 推荐采用的 POX 染色液，其原理是细胞内的过氧化物酶能将无色的 DAB 的氢原子传递给过氧化氢，使前者催化成有色染料沉积在细胞质中的 POX 所在部位。该染液可用于血液、骨髓或细胞涂片过氧化物酶染色, POX 活性部位呈棕黄色。

自备材料：

载玻片、显微镜

操作步骤：(*仅供参考*)

1、 血液、骨髓或细胞涂片滴加预冷的BFA固定液，4℃固定30-60s，稍水洗。
2、 滴加配制好的 POX 孵育液，室温(20-25℃)避光孵育 10-15min，水洗 2min。
3、 滴加 WG 染色液，孵育 30-60s。
4、 直接滴加等量 WG 缓冲液，染色 10-15min。
5、 水洗、晾干、镜检。

染色结果：

POX 活性部位	棕黄色
细胞核	蓝色

粒细胞系除早期原粒细胞阴性外，分化好的原粒细胞以下阶段细胞随细胞成熟而阳性反应增强，衰老中性粒细胞反应程度减弱，单核细胞系弱阳性，淋巴细胞系为阴性。浆细胞及巨核细胞均为阴性。嗜酸性粒细胞和 Auer 小体呈强阳性反应。

阳性反应强度的判断：

阴性	无颗粒
弱阳性	颗粒小，分布稀疏
阳性	颗粒粗略，分布密集
强阳性	颗粒粗大，呈蓝黑色，充满胞浆

临床意义：

1、 急性粒细胞白血病晚期的原粒细胞呈阳性，颗粒较少且大。
2、 急性单核白血病细胞呈阴性或弱阳性，颗粒小且稀疏。
3、 单核急性白血病呈阴性或弱阳性。
4、 急性早幼粒白血病呈强阳性，某些早幼粒细胞呈阳性，恶性组织细胞呈阴性。

5、 急性淋巴细胞白血病呈阴性。

注意事项：

1、 血液或骨髓涂片应新鲜，薄厚适宜，及时固定，否则会影响酶的活性。
2、 POX孵育液易失效或降低阳性强度，即配即用，不宜久置。
3、 样本在未染色前切勿接触氧化剂类物质，以免细胞内的过氧化物酶被抑制。
4、 每次染色时，应采取健康人末梢血或骨髓涂片作为阴性对照。
5、 为了您的安全和健康，请穿实验服并戴一次性手套操作。

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Peroxidase Stain Kit(DAB Method)

Cat: G2370
Size:4×10mL/4×20mL
Storage:-20℃, avoid light, valid for 1 year.

Kit Components

Reagent		4×10mL	4×20mL	Storage
Reagent (A): BFA Fixative		10mL	20mL	2-8℃, avoid light
Reagent (B): POX Incubation Solution	B1: DAB Solution	10mL	20mL	-20℃, avoid light
	B2: DAB Oxidant	2×100μL	4×100μL	2-8℃, avoid light
Mix B1with B2 as the radio of 1000:1 to form POX Incubation Solution. It is ready to use.				
Reagent (C): WG Solution		10mL	20mL	RT, avoid light
Reagent (D): WG Buffer		10mL	20mL	RT

Introduction

Peroxidase (POX or MPO) is a kind of oxidoreductase produced by microorganisms or plants, which can catalyze many reactions and catalyze the oxidation of the substrate with hydrogen peroxide as the electron acceptor. It mainly exists in the peroxisome of cells. With iron porphyrin as the auxiliary group, it can catalyze the oxidation of phenols and amines by hydrogen peroxide. It has double effects of eliminating the hydrogen peroxide, phenols and amines toxicity.

Peroxidase Stain Kit (DAB Method) is the POX solution recommended by ICSH. Its principle is that the peroxidase in the cell can transfer the hydrogen atom of the colorless DAB to hydrogen peroxide, so that the former catalyzes the formation of colored dye and deposits it in the position of pox in the cytoplasm. The dye can be used for peroxidase staining of blood, bone marrow or cell smear. The active part of pox is brown.

Self Provided Materials

Slide, Microscope.

Protocol(*for reference only*)

1. Add precooled BFA Fixative onto the blood, bone marrow or cell smear and fix at 4 ℃ for 30-60s , then wash slightly.
2. Add the prepared POX Incubation Solution and incubate at room temperature (20-25 ℃) in dark for 10-15 mins, then wash with water for 2 mins.
3. Add WG Solution and incubate for 30-60s.
4. Add equal amount of WG Buffer directly and dye for 10-15 mins.
5. Wash with water, dry and view under the microscope.

Result

POX Active Site	Brown Yellow
Nucleus	Blue

In addition to the early neutrophil negative, the positive reaction increases with the maturation of the well-differentiated cells in the following stages, while the senescent neutrophils decreases, monocyte is weak positive and lymphocyte is negative. Plasma cells and megakaryocytes are negative. Eosinophils and Auer bodies show strong positive reaction.

Judgment of positive reaction intensity

Negative	No particle
Weakly Positive	Small particles with sparse distribution
Positive	Coarse particles with dense distribution
Strongly Positive	The particles are coarse, blue black and full of cytoplasm.

Clinical significance

1. In the late stage of acute myeloid leukemia, there are few and large granules.
2. Acute monocytic leukemia cells are negative or weakly positive, there are small and sparse granules.
3. Monocytic acute leukemia is negative or weakly positive.
4. Acute promyelocytic leukemia is strongly positive, some promyelocytes are positive, and malignant cells are

negative.
5. Acute lymphocytic leukemia is negative.

Note

1. Blood or bone marrow smear should be fresh, thin and appropriate, fix in time, otherwise it will affect enzyme activity.
2. POX Incubation Solution is easy to lose efficacy or reduce the positive intensity. It is ready to use and not stored for long time.
3. Do not touch oxidants before the sample is stained to avoid the inhibition of peroxidase in cells.
4. For every staining, the smear of peripheral blood or bone marrow should be taken as negative control.
5. For your safety and health, please wear experimental clothes and disposable gloves.